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LETTER TO THE EDITOR

Ustekinumab demonstrates activity in glucocorticoid-refractory acute GVHD

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Novel approaches are needed for GVHD prophylaxis and therapy. Naive CD4+ T cells differentiate into distinct lineages (Th1, Th2, Th17, Treg) under the influence of APCs and specific cytokine programs. Data suggest unique contributions of these CD4+ T-cell subsets to the target manifestations of GVHD.[1] Donor Th1 CD4+ T cells have a central role in the pathogenesis of acute GVHD, and preclinical data have demonstrated that neutralization of IL-12 prevented the development of acute GVHD, polarized CD4+ cells toward a Th2 phenotype and provided long-term protection from GVHD in mice.[2] Th17 cells have been implicated in solid organ allograft rejection and autoimmunity, and emerging evidence demonstrates their role in the pathogenesis of acute GVHD. In murine transplantation models, Th17 cells infiltrated target organs and were sufficient for the generation of GVHD.[3–5] In addition, secretion of IL-23 by APCs is an essential component of GVHD induction,[6] indicating the relevance of this cytokine, in particular, as a therapeutic target. Thus, inhibition of both Th1 and Th17 CD4 differentiation programs may provide a powerful novel approach to GVHD control.[2–10] Ustekinumab (Stelara; Centocor Inc, Malvern, PA, USA) is a human, Ig G1-κ MoAb, which binds the p40 subunit shared by the cytokines IL-12 and IL-23. Given the activity of this agent in allied immune-mediated disorders, there is a rationale to neutralize p40 in the prevention or therapy of GVHD.

A 39-year-old woman with severe aplastic anemia underwent an unrelated (mismatched at one HLA-A allele) transplant on a phase II clinical protocol of GVHD prevention, using humanized anti-CD3 Ab visilizumab, tacrolimus and MTX (clinicaltrial.gov NCT00720629). By day +28 post HCT, she had biopsy-confirmed acute GVHD, covering approximately 70% body surface area, which was refractory to 2 mg/kg/day of prednisone, topical triamcinolone 0.1% cream and multiple lines of rescue therapy (Figure 1a). Beyond the first line therapy with systemic and topical glucocorticoids and continuation of tacrolimus, the following additional systemic immune suppressive therapies were utilized in an effort to achieve GVHD control: sirolimus (week 3 from GVHD onset, maintained at therapeutic serum levels thereafter); extracorporeal photopheresis (week 3, thereafter performed biweekly for duration of follow-up); mycophenolate mofetil (started on week 6, continued thereafter); and psoralen and ultraviolet A therapy (PUVA) (started on week 6, performed three times weekly thereafter). During week 19 of ongoing GVHD therapy, she was treated with ustekinumab 45 mg s.c. injection. This dose was chosen on the basis of the approved dose for psoriasis. In the 4 weeks of clinical observation that followed this therapy, CR of acute GVHD was achieved. Unfortunately, the patient expired during week 23 from *Escherichia coli* septicemia and pneumonia.

Correlative studies were performed on the patient's samples 70 days before and 35 days following ustekinumab therapy. Serum cytokine levels, including total p40 (free or Ab-bound), were determined by ELISA assay (Quansys Biosciences, Logan, UT, USA). Following ustekinumab, circulating serum IL-12p40 was increased (Figure 1b). Serum IL-2, IL-4, IL-5, IL-10, IL-17, IL-23, IFN-γ, TNF-α were below detectable range, both pre- and post-therapy by this method. Absolute CD4+, CD8+ and Treg (CD4+, CD25+, CD127low) numbers were quantified from whole blood. CD4+ non-Tregs (165–19 cells/μL), CD8+ T cells (48–18/μL) and Tregs (2.09–1.37/μL) all decreased follow-

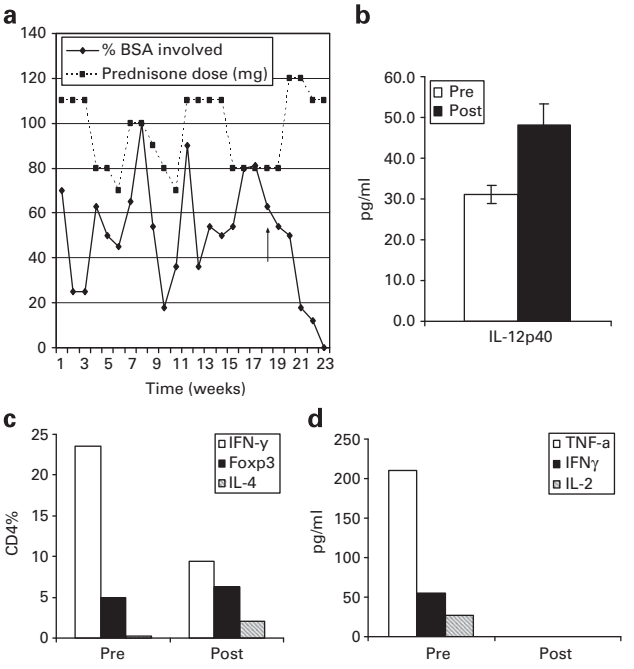

Figure 1 (**a**) Clinical course of acute GVHD activity and associated therapy, (**b**) serum IL-12p40 level by ELISA, (**c**) cell phenotype analysis following PMA/ionomycin stimulation, and (**d**) supernatant cytokine analysis following PMA/ionomycin stimulation. (**a**) Prednisone dose represented in total mg dose (corresponded to 1.2–2 mg/kg body weight over course of therapy). Additional rescue agents delivered for glucocorticoid-refractory acute GVHD were initiated at the following time points: sirolimus (week 3), extra-corporeal photopheresis (week 3), mycophenolate mofetil (week 6), PUVA (week 6) and ustekinumab (week 19, indicated by arrow). (**b**–**d**) Pre- (70 days before ustekinumab) and post- (35 days following ustekinumab) samples.

ing ustekinumab. To assess T-cell cytokine profile, PBMCs were activated with phorbol IZ-myristate 13-acetate (PMA) (50 ng/mL), Ionomycin (500 ng/mL) and Golgi stop (BD Biosciences, San Jose, CA, USA; 0.7 μL/mL), and cultured at 37 °C, 5% CO_2 for 5 h. Cells were then washed and stained with CD3, CD4, CD8, CD25, CD127, Foxp3, IL-4, IL-17 and IFN-γ antibodies. Although IFN-γ-positive cells decreased following therapy, those positive for FoxP3 and those producing IL-4 increased (Figure 1c). In the analysis of supernatant cytokines, PBMC were activated with PMA/Ionomycin as above. TNF-α, INF-γ and IL-2 decreased, whereas IL-4, IL-5, IL-10, IL-17 and TGF-β were not detectable (Figure 1d).

In this case of advanced, refractory acute GVHD, we report that a single s.c. dose of ustekinumab was successful in inducing clinical remission of GVHD activity. The response to this agent was notable, given the advanced GVHD syndrome and failure of multiple lines of immune suppressive therapy. Alongside this clinical activity, correlative data appears to support the skewing of the CD4+ T-cell repertoire, given the marked reduction in Th1 phenotype and cytokines, and the increase in Th2 and Treg phenotype following ustekinumab therapy. Taken together, this early evidence supports the notion that targeting IL12p40 may be relevant to the differentiation of pathogenic Th1, and perhaps Th17 CD4+ T cells, may interrupt acute GVHD pathobiology and result in clinical control of the syndrome. Although this report is intriguing and provides the first evidence of the clinical activity of this agent in the management of acute GVHD, we acknowledge several limitations. First, as is the case with any salvage agent in therapy of refractory acute GVHD, it is difficult to discern the true activity of ustekinumab in the setting of multiple other administered immune suppressive therapies. Although response was not achieved with the prior agents, it is speculative to assign the ultimate response to the agent in question alone. Next, the patient unfortunately expired, shortly after achieving CR of GVHD activity, and therefore, the durable impact of this therapy in GVHD control can not be discerned from this limited experience. Assessment of cell subsets and cytokine elaboration was limited to the available planned samples obtained according to the clinical visilizumab protocol as well. Samples obtained in closer proximity to the delivery of ustekinumab could provide more informative data, but this was not possible. Finally, this correlative data is restricted only to peripheral blood and serum samples, and we therefore cannot speak to associated findings in GVHD target organs. We acknowledge that immune suppressive therapies, including monoclonal antibodies such as ustekinumab, may impair beneficial immune responses associated with control of infection and malignancy as well. Recognizing these limitations, the data suggest the potential activity of ustekinumab in the control of established acute GVHD, and support a rationale for the use of this agent for GVHD prevention.

Conflict of interest

The authors declare no conflict of interest.

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